

## VENTILATION IN AN AQUATIC AND A TERRESTRIAL CHELONIAN REPTILE

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### SUMMARY

1. Periods of breath-holding are interrupted by episodes of continuous breathing in the aquatic turtle *Pelomedusa subrufa*, whereas single breaths and short periods of breath-holding alternate in the terrestrial tortoise *Testudo pardalis*. This implies that partial pressures of O<sub>2</sub> and CO<sub>2</sub> in expired air are stable in *Testudo* in contrast to cyclic fluctuations in *Pelomedusa*.

2. In spite of this, air convection requirements ( $\dot{V}_A/\dot{V}_{O_2}$ , ml BTPS/ml STPD) are not significantly different for the two species (25.4 in *Testudo*, 27.3 in *Pelomedusa*), and differences in weight-specific ventilation between the species can only be ascribed to a difference in mean body weight.

3. The end-tidal  $P_{CO_2}$  in *Pelomedusa* (mean 15.2 mmHg) is lower than in *Testudo* (mean 24.7 mmHg), which reflects aquatic CO<sub>2</sub> elimination in *Pelomedusa*.

4. In *Testudo*, the time course of ventilation correlates with the time course of increase of end-tidal  $P_{CO_2}$  during CO<sub>2</sub> breathing, but no simple relationship is evident between ventilation and blood  $P_{CO_2}$  in *Pelomedusa*.

5. In both species tidal volume as well as respiratory frequency increase approximately in proportion to the end-tidal  $P_{CO_2}$ , although the response to 6% CO<sub>2</sub> breathing could be less than expected from extrapolation of the responses to 2 and 4% CO<sub>2</sub>. Both species also increase ventilation during hypoxia, but hyperoxia depresses ventilation.

### INTRODUCTION

The tortoise *Testudo* and the turtle *Pelomedusa* exhibit two different modes of breathing. Periods of breath-holding are typical of the normal breathing pattern in both species, but such periods are interrupted by episodes of continuous breathing in *Pelomedusa*, whereas single breaths and short periods of breath-holding alternate in *Testudo*. The periodic breathing pattern of *Pelomedusa* is typical of aquatic reptiles, and persists both in *Pelomedusa* and in other species when they leave the water (Burggren, 1975).

Periodic ventilation suggests that  $P_{O_2}$  and  $P_{CO_2}$  in both the alveolar gas and blood

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will change in a cyclic manner (Lenfant *et al.* 1970). The fluctuations in blood gas tensions in chelonians are also complicated by intracardiac blood shunting. A net left-to-right shunt normally occurs during the period of ventilation, and a net right-to-left shunt of blood away from the lungs increases during apnoea (Millen *et al.* 1964; White & Ross, 1965, 1966; Johansen, Lenfant & Hanson, 1970; Shelton & Burggren, 1976).

Nearly every study on the control of ventilation in reptiles during hypoxia or hypercapnia has been deficient in measurements of blood and alveolar  $P_{O_2}$  and  $P_{CO_2}$  (Wood & Lenfant, 1976). Exceptions are the studies on control of ventilation in the freshwater turtle, *Pseudemys scripta elegans*, in which blood gas tensions were measured (Frankel *et al.* 1969; Jackson, Palmer & Meadows, 1974). However, measurements in these studies did not provide information on the time needed for the arterial  $P_{O_2}$  or  $P_{CO_2}$  to attain stable values during hypoxia or hypercapnia.

The present paper deals with the relationship of ventilation to lung (end-tidal)  $P_{CO_2}$  in two chelonian species, the terrestrial tortoise *Testudo pardalis*, and an aquatic species, the side-necked turtle *Pelomedusa subrufa*. The time course for development of the ventilatory response during hypercapnia was measured in both species. This was compared with the time course of increase of end-tidal  $P_{CO_2}$  in *Testudo*, while rate of increase of blood  $P_{CO_2}$  was obtained for *Pelomedusa*. The effect of changing inspired  $P_{O_2}$  on ventilation was measured in both species and reference values on the normal patterns of breathing were obtained. Air convection requirements were also studied. The air convection requirement ( $\dot{V}_A/\dot{V}_{O_2}$ ) of an animal is the volume of air which must ventilate the gas exchange surfaces to extract a unit amount of oxygen (Dejours, 1972).

#### METHODS

Four leopard tortoises, *Testudo pardalis*, and five side-necked turtles, *Pelomedusa subrufa*, were airfreighted from Kenya to Aarhus, Denmark. The tortoises weighed from 2 to 3.5 kg (mean 2.9 kg), and the turtles from 0.3 to 0.8 kg (mean 0.57 kg). The tortoises were fed a varied diet of fresh vegetables, whereas the turtles were offered liver and fish meat. The animals readily accepted the food and were in excellent condition. Animals were not fed for 2 days prior to experiments. All measurements were performed at 25 °C, which is within the range of the preferred temperatures for both species, and the animals were kept at that temperature between experiments.

#### *Measurements of ventilation*

A skin-tight mask of flexible polymer was constructed for each animal (see Glass, Wood & Johansen, 1977, for details). The mask was glued to the head of the animal using a rapidly setting epoxy glue. Mask and glue covered the front of the head and the mouth, but the eyes were unobstructed. A light-weight Fleisch head (Fleisch, 1925) connected to a Godart pneumotachograph was inserted into an opening in the mask over the nostrils. Calibration was performed as advised by Hobbes (1967). The mask with the inserted Fleisch head did not prevent the animal from retracting its head.

During experiments the animals were placed in a plexiglass chamber which per-

mitted observation of the animal. The outlet tube served additionally for passing the pressure and gas sampling tubes from the face mask to pneumotach and gas analysers. The dimensions of the chamber restricted movements of the animal, but did not interfere with its natural posture.

When ventilation of a *Pelomedusa* was measured the experimental chamber was partly filled with water, but only to a level at which the animal was able to submerge its head. Unlike *Testudo*, which readily tolerated face masks, it took several days to condition a *Pelomedusa* to wear a mask. Ventilation was measured in two different ways for this reason. The alternative was the buoyancy technique described by Jackson (1971). With this technique, the turtle was suspended by strings from a Grass strain gauge transducer. The body was lowered into water, but the turtle could raise its head to the surface for breathing. Lung inflation and deflation caused changes in the buoyancy of the animal which were monitored by the strain gauge transducer and a recorder (Brush 260). The accuracy of this method of measuring tidal volume is very high and is set by the sensitivity of the strain gauge transducer. Its successful application, however, depends on minimum restraint of the turtles since the mechanics of ventilation require relatively free leg movements. Fortunately the *Pelomedusa* were usually quiescent during measurements and only needed to be loosely restrained. The two methods of measuring ventilation were in excellent agreement, differing by less than 5%.

#### *Measurement of partial pressures of inspired and expired gases*

CO<sub>2</sub> contents of gases were measured by a Beckman LB2 CO<sub>2</sub> analyser which was calibrated with a Wösthoff gas mixing pump fed with pure gases. The sampling rate of the LB2 was reduced to about 60 ml.min<sup>-1</sup>. This reduction increased the lag and rise time of the LB2, but it was possible in every case to obtain an 'alveolar plateau' during expiration. The sample cell of the LB2 was frequently cleaned to assure linear performance. Expired gas was sampled on a breath-to-breath basis through a catheter terminating in a hypodermic needle that penetrated the mask close to the nostrils. Sampling from this point minimized dead space.

P<sub>O<sub>2</sub></sub> of hypoxic gases was measured by means of a Radiometer O<sub>2</sub> electrode mounted in a cuvette. The P<sub>O<sub>2</sub></sub> was continuously displayed on a Radiometer O<sub>2</sub> module. P<sub>O<sub>2</sub></sub> of a single breath could not be measured by the O<sub>2</sub> electrode, but was measured by a Medspect 2 respiratory mass-spectrometer, which was used in ten experiments to obtain end-tidal P<sub>O<sub>2</sub></sub> and P<sub>CO<sub>2</sub></sub>. Information on both gases is needed for the calculation of the air convection requirements.

#### *Preparation of gas mixtures*

Hypercapnic or hypoxic gas mixtures were prepared by feeding gases from cylinders through Fisher and Porter flowmeters into a water-filled wash bottle where mixing took place. The experimental chamber was continuously flushed with the mixture at a rate of 2-3 l.min<sup>-1</sup>. The partial pressures of CO<sub>2</sub> in the test gases were 14.7, 29.4 and 44.2 mmHg corresponding to 2, 4 and 6% of the dry gas content at 25 °C and 1 atmosphere. Partial pressures of the hypoxic or hyperoxic gases were 37, 74, 155 and 736 mmHg at the same temperature and barometric pressure.

### Measurement of blood gases

The methods of intravascular cannulation were described in detail by Shelton & Burggren (1976) and Burggren, Glass & Johansen (1977). The common pulmonary artery and the left subclavian artery were non-occlusively cannulated in an upstream direction. Sampling of blood was not started until 24 h after surgery. The blood samples were analysed with Radiometer equipment (BMS 3, PHM 71). The total accumulated volume from repeated sampling ranged between 3.3 and 5.1 ml/kg.

### Experimental protocol

An animal was left undisturbed in the experimental chamber for 4–7 h (mean 5 h) after glueing of mask and Fleisch head to the animal. Test gases were introduced when a stable minimum ventilation had been observed. Composition of the inspired gas could be altered within half a minute. Inhalation of the test gas usually lasted about 1 h. The animal was allowed to recover in atmospheric air before another test gas was introduced.

### Definitions and calculations

The peak  $P_{\text{CO}_2}$  of the expired gas is the end-tidal  $P_{\text{CO}_2}$  ( $P_{\text{ET CO}_2}$ ), which was used as an approximation to the mean alveolar  $P_{\text{CO}_2}$ . All gas rates are calculated as  $\text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ , but conventionally the inspired minute volume ( $\dot{V}_I$ ) and the alveolar minute volume ( $\dot{V}_A$ ) are at body temperature and pressure, saturated with water vapour (BTPS), whereas volumes of  $\text{O}_2$  and  $\text{CO}_2$  are calculated for standard conditions (STPD = 0 °C, 760 mmHg, dry). The ratio  $\dot{V}_A/\dot{V}_{\text{CO}_2}$  was calculated by means of the equation  $\dot{V}_A/\dot{V}_{\text{CO}_2} = R \cdot T/P_A \text{CO}_2$  where  $R$  is the gas constant equal to 2.785  $\text{ml} \cdot \text{mmHg} \cdot ^\circ\text{K}^{-1} \cdot \text{ml}^{-1}$  STPD. Air convection requirements could be calculated from the respiratory quotient of the expired air and the ratio  $\dot{V}_A/\dot{V}_{\text{CO}_2}$ . Expired volumes were less than inspired volumes in *Pelomedusa* due to  $\text{CO}_2$  elimination to the water. The ventilations discussed in the following pages are based on inspired volumes ( $\dot{V}_I$ ), since expired volumes are not directly comparable in *Pelomedusa* and *Testudo* due to a marked difference in their pulmonary gas exchange ratios.

## RESULTS

### Normal stable breathing

Fig. 1 shows recorded tracings of representative breathing patterns of *Testudo* and *Pelomedusa*. Periods of breath-holding and single breaths alternated in *Testudo*, whereas breathing in *Pelomedusa* was characterized by episodes of several consecutive breaths separated by periods of breath-holding. In both species breath-holding was preceded by an inspiration and resumption of breathing was initiated by an expiration. A ventilatory period in *Pelomedusa* usually consisted of four to seven breaths, but breath-holding was of variable duration (mean of five animals being 4.3 min.) (Fig. 8). This is about six times the mean interval between breaths for four *Testudo* (0.7 min). Table 1 compares tidal volume, respiratory frequency and ventilation in both species. It is based on periods that include about 50 breaths and thus several episodes of ventilatory activity for each animal. Therefore, the frequencies express long-term values and do not reflect the temporal spacing between individual breaths.

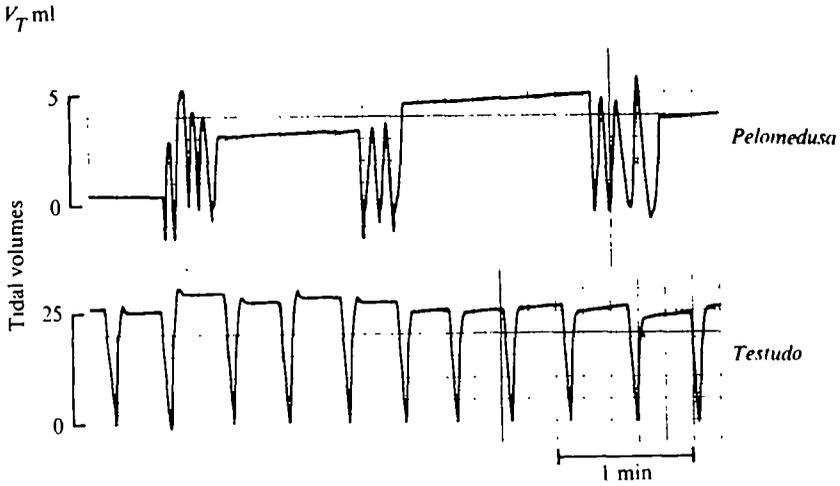


Fig. 1. Typical patterns of breathing in *Testudo* and *Pelomedusa*.

Table 1

*Testudo pardalis* (n: 4 animals); 25 °C

	$\bar{x}$	S.E.	Range
Weight (kg)	2.9	0.3	2.1-3.4
$f$ (breaths . min <sup>-1</sup> )	1.2	0.9	0.5-4.2
$V_T$ (ml BTPS . kg <sup>-1</sup> )	10.7	2.7	4.7-18.0
$\dot{V}_I$ (ml BTPS . kg <sup>-1</sup> . min <sup>-1</sup> )	13.4	3.6	4.3-19.7
$\dot{V}_A/\dot{V}_{O_2}$ (ml BTPS/ml STPD)	25.4	1.0	22.9-27.7
$P_{ETCO_2}$ (mmHg)	24.7	1.2	22.5-27.2
R	0.76	0.02	0.73-0.81

*Pelomedusa subrufa* (n: 5 animals); 25 °C

	$\bar{x}$	S.E.	Range
Weight (kg)	0.6	0.1	0.3-0.8
$f$ (breaths . min <sup>-1</sup> )	1.4	0.2	0.8-2.1
$V_T$ (ml BTPS . kg <sup>-1</sup> )	15.5	2.4	11.1-22.2
$\dot{V}_I$ (ml BTPS . kg <sup>-1</sup> . min <sup>-1</sup> )	22.4	5.6	7.0-41.3
$P_{aO_2}$ (subcl. art.) (mmHg)	70.0	6.3	52.5-82.3
$P_{vO_2}$ (pulm. art.) (mmHg)	33.7	0.7	32.3-34.5
$P_{aCO_2}$ (subcl. art.) (mmHg)	18.3	1.2	15.8-21.5
$P_{vCO_2}$ (pulm. art.) (mmHg)	20.5	1.1	18.5-22.6
$(\dot{V}_A/\dot{V}_{O_2})$ (ml BTPS/ml STPD)	27.3	(mean of two animals)	
$P_{ETCO_2}$ (mmHg)	15.2	(mean of two animals)	
R	0.52	(mean of two animals)	

Abbreviations used:  $V_T$  = tidal volume;  $\dot{V}$  = volume of gas per unit time;  $I$  = inspired;  $A$  = alveolar;  $P$  = pressure or partial pressure;  $ET$  = end-tidal;  $\bar{v}$  = mixed venous;  $a$  = arterial.

The table also shows end-tidal  $P_{CO_2}$ , respiratory quotients, and air convection requirements for both species and in addition blood gas tensions in *Pelomedusa*. It is evident from the table that overall respiratory frequencies of the two species were not significantly different in spite of the differences in breathing pattern. Frequency was only slightly above 1 breath/min in both species. The mean weight-specific tidal volume in *Pelomedusa* (15.5 ml/kg) was larger than in *Testudo* (10.7 ml/kg), but the variation

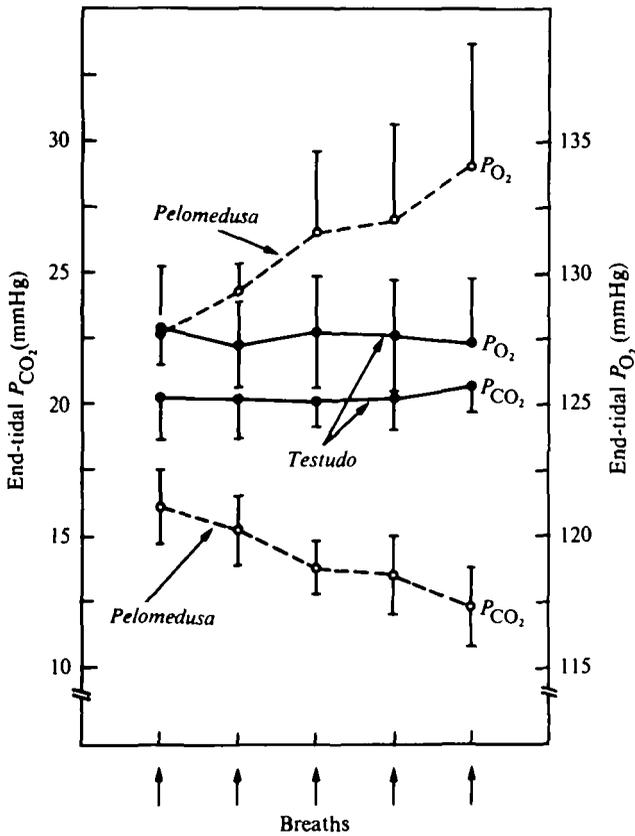


Fig. 2. End-tidal  $P_{O_2}$  and  $P_{CO_2}$  in a *Testudo* and a *Pelomedusa*. The partial pressures change progressively during ventilatory periods in *Pelomedusa*, whereas repeated sequences of expirations in *Testudo* show a stable  $P_{O_2}$  and  $P_{CO_2}$ . Mean values  $\pm 1$  s.d.

between the individual animals was large in both species. The mean end-tidal  $P_{CO_2}$  in *Pelomedusa* was two-thirds of that in *Testudo* (Fig. 2). The  $P_{ETCO_2}$  and  $P_{ETO_2}$  in the tortoise were very stable when breathing was regular. In contrast the periodic mode of breathing in *Pelomedusa* caused a progressive decrease of  $P_{ETCO_2}$  and increase of  $P_{ETO_2}$  with each breath during ventilation (Fig. 2). The mean respiratory quotient calculated on the basis of expired air composition was 0.76 in *Testudo* but only 0.52 in *Pelomedusa*. The latter value reflects aquatic  $CO_2$  elimination which is of variable importance in aquatic turtles (Belkin, 1963). Air convection requirements were not significantly different in *Pelomedusa* (27.3 ml BTPS/ml STPD) and *Testudo* (25.4 ml BTPS/ml STPD) in spite of the differences in patterns of breathing. The importance of the blood gas levels in normal control of breathing was difficult to assess both because of the small number of samples and their arbitrary timing in relation to the breathing cycle. Subclavian arterial  $P_{O_2}$  was approximately 50 mmHg higher than pulmonary arterial  $P_{O_2}$  in all *Pelomedusa*, a situation similar to that in other chelonian reptiles with a functionally undivided ventricular pump (Burggren, 1976).  $P_{aCO_2}$  averaged 18.3 mmHg, compared with 23.3 mmHg in the pulmonary artery (Table 1).

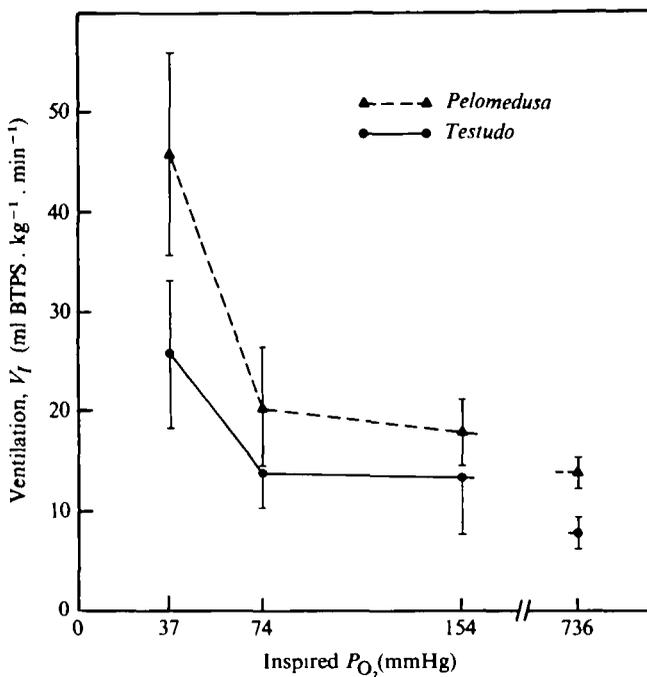


Fig. 3. Relationship between inspired  $P_{O_2}$  and ventilation in *Pelomedusa* and *Testudo*. Mean values  $\pm$  1 S.E.

### Hypoxia and hyperoxia

Ventilation was increased by hypoxia and depressed by hyperoxia in both species (Fig. 3). Lowering of the inspired  $P_{O_2}$  from normal (about 155 mmHg) to 37 mmHg increased ventilation by a factor of 2.9 in *Pelomedusa* and 1.9 in *Testudo*. The responses were produced by variable changes in both tidal volume and frequency, but a consistent change of these variables was only observed in two cases. One *Pelomedusa* consistently increased frequency when  $P_{I O_2}$  was decreased from 736 to 37 mmHg, whereas a tortoise consistently increased tidal volume. The end-tidal  $P_{O_2}$  in the tortoises decreased to 25–28 mmHg, when  $P_{I O_2}$  was 37 mmHg. The same  $P_{I O_2}$  caused the  $P_{a O_2}$  in *Pelomedusa* to drop from the normal average value of 70 mmHg to 31 mmHg (range: 25–35 mmHg), while the average  $P_{O_2}$  of the pulmonary artery dropped from 33.7 to 22.3 mmHg (range: 16–29 mmHg).

### Hypercapnia

The time course of the ventilation increase during hypercapnia in *Testudo* was correlated with the rate of increase of end-tidal  $P_{CO_2}$ , but the ventilation increase in *Pelomedusa* was not in simple proportion to blood  $P_{CO_2}$ . Fig. 4 shows the time course of the increase of end-tidal  $P_{CO_2}$  and ventilation during hypercapnia for three specimens of *Testudo*. The increases are shown as percentages of the maximum increases. Mean  $P_{ET CO_2}$  was 33 mmHg but still increased slightly, even after nearly an hour of  $CO_2$  breathing. The increase of ventilation reached a maximum (mean 206 ml  $\cdot$  kg $^{-1}$   $\cdot$  min $^{-1}$ ) after about 40 min. The time course of blood  $P_{CO_2}$  and the time-

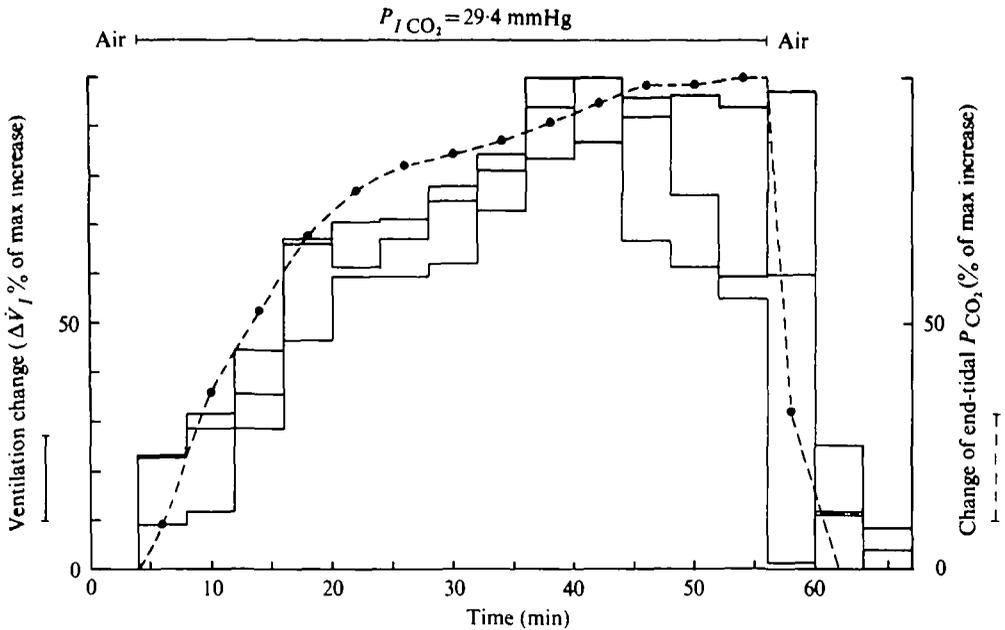


Fig. 4. Time course of increase of end-tidal  $P_{CO_2}$  and ventilation during hypercapnia in three specimens of *Testudo*. The change of ventilation is presented as step functions for the individual animals, whereas change of end-tidal  $P_{CO_2}$  is shown as a continuous function based on point measurements and mean values.

response characteristics of ventilation during hypercapnia are shown in Fig. 5 for three specimens of *Pelomedusa*. Changes in ventilation are averaged for 10 min periods to eliminate the irregularities caused by periodic ventilation, which persisted even during hyperventilation.  $P_{CO_2}$  in the pulmonary artery and the left subclavian artery increased steadily during all 30 min of the experiment, but on the average 85% of the ventilation increase occurred during the first 10 min of the experiment, with a maximum at 10–20 min after the start of  $CO_2$  breathing.

Fig. 6 shows the difference between end-tidal  $P_{CO_2}$  and inspired  $P_{CO_2}$  plotted against inspired  $P_{CO_2}$  for four specimens of *Testudo*. The differences are those that occur after 40 min of  $CO_2$  breathing, and are much reduced at high inspired  $P_{CO_2}$ .

The relationships between end-tidal  $P_{CO_2}$ , tidal volume, respiratory frequency, and ventilation are shown in Figs. 7–9. The low  $P_{ETCO_2}$  values are those of the normal stable breathing pattern. The increases of ventilation are induced by inspiration of 2, 4 and 6%  $CO_2$  (dry gas content). The figures are based on the conditions during maximum response to the test gases. The responses of the individual animals are presented for *Testudo*. Tidal volume and frequency increased in proportion to end-tidal  $P_{CO_2}$ , up to about 35 mmHg, but the effect of increasing  $P_{ETCO_2}$  to about 45 mmHg was less than would be expected from an extrapolation of the response to an increase of  $P_{ETCO_2}$ , up to 35 mmHg. The frequency increase was less consistent than the increase of tidal volume. The increase of ventilation per unit increase of  $P_{ETCO_2}$  ( $ml\ BTPS \cdot kg^{-1} \cdot min^{-1} \cdot mmHg^{-1}$ ) ranged widely, from 4.7 to 35.1.

The responses of *Pelomedusa* are presented as grouped data and include measure-

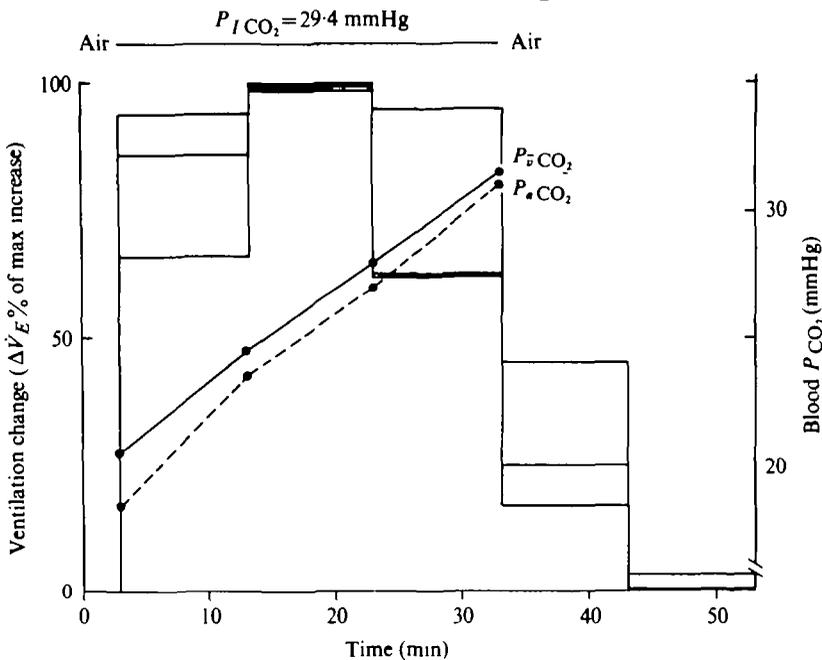


Fig. 5. Ventilation change and increase of blood  $P_{\text{CO}_2}$  during hypercapnia in *Pelomedusa*. Change of ventilation is shown as a step function for each animal, but blood  $P_{\text{CO}_2}$  is a mean value for the three specimens.

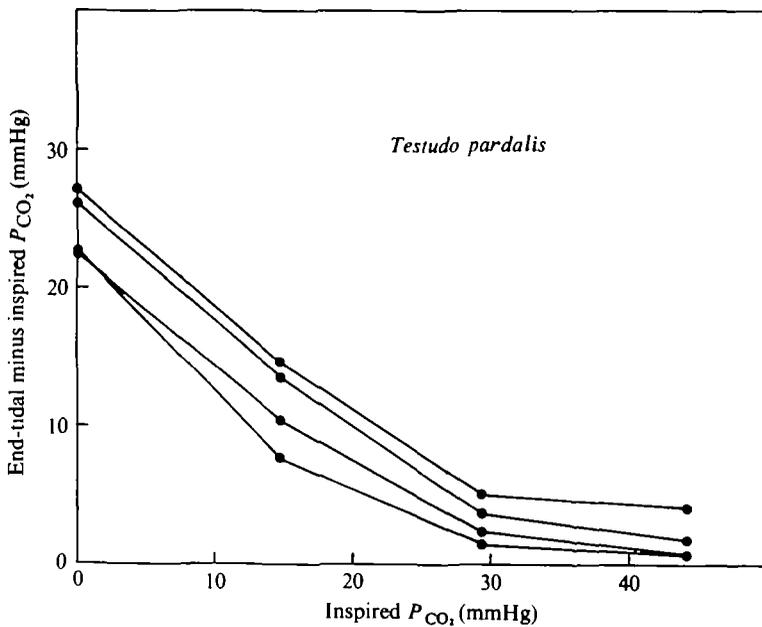


Fig. 6. End-tidal  $P_{\text{CO}_2}$  minus inspired  $P_{\text{CO}_2}$  versus inspired  $P_{\text{CO}_2}$  for four specimens of *Testudo*.

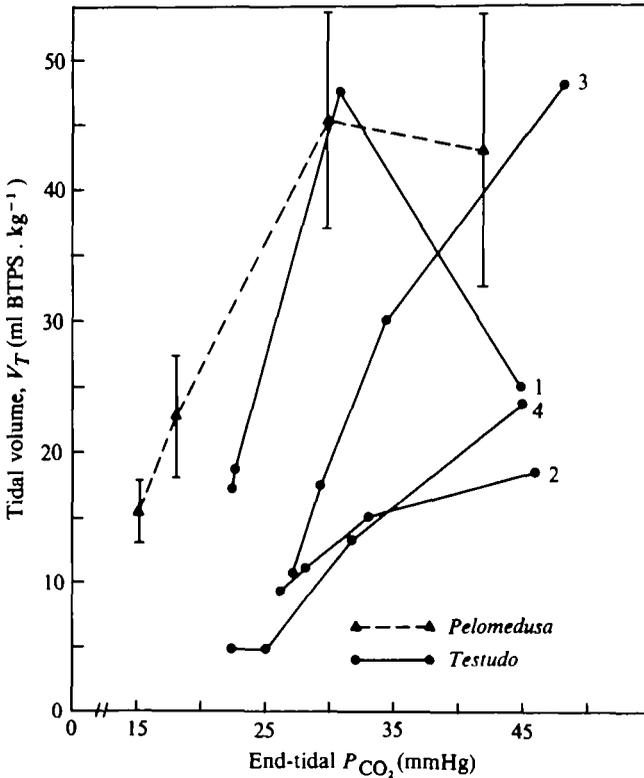


Fig. 7. Relationship between end-tidal  $P_{CO_2}$  and tidal volume in *Testudo* and *Pelomedusa*. The responses of the individual animals are shown for *Testudo*, but the data on *Pelomedusa* are presented as mean values  $\pm 1$  S.E.

ments of ventilation in five specimens; expired  $P_{CO_2}$  was measured in two of these. The  $P_{ETCO_2}$  is an average including all expirations. Both tidal volume, frequency, and the resulting ventilation appear to increase approximately in proportion to the end-tidal  $P_{CO_2}$ , up to a value of 30 mmHg. These relationships would not result if the mean  $P_{ETCO_2}$  of all expirations was replaced by the mean value for the expirations that initiate the ventilatory periods. This mean value is 17 mmHg during normal stable breathing, which would shift the points for the normal breathing pattern to the right in Figs. 7–9. The other points would remain unchanged, because hypercapnia practically abolished the variation in end-tidal  $P_{CO_2}$ . It is worth noting that when  $P_{ICO_2}$  was increased to 44 mmHg the end-tidal  $P_{CO_2}$  of *Pelomedusa* was less than inspired  $P_{CO_2}$ . This reflects  $CO_2$  elimination to the water.

In *Pelomedusa*, inspiration of 2%  $CO_2$  reduces the duration of the breath-holding periods to one-third of the initial mean value. Notably a much shortened breath-holding period persists at the 6%  $CO_2$  level. By comparing Figs. 8(a) and (b), it is evident that the shortening of the breath-holding periods alone can not account for the frequency response to hypercapnia. The breathing frequency and the duration of the ventilatory periods are also modified.

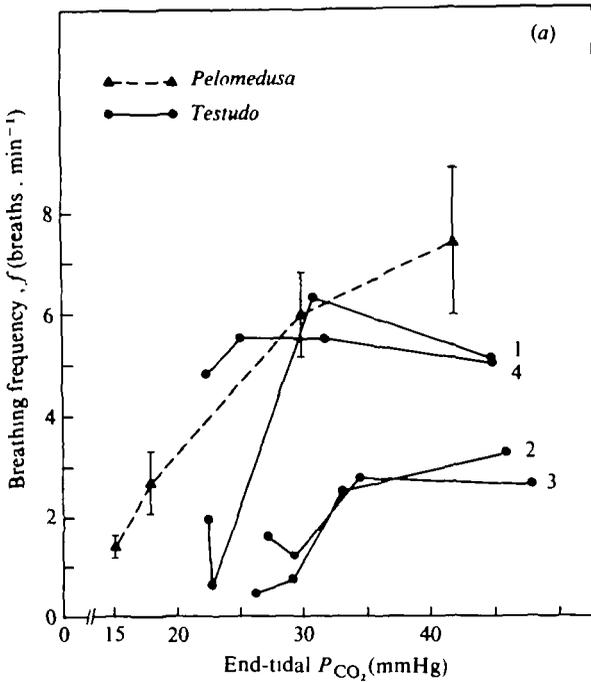


Fig. 8 (a). Relationship between end-tidal  $P_{CO_2}$  and respiratory frequency in *Testudo* and *Pelomedusa*. The combination of animals and the symbols used are the same as in Fig. 7.

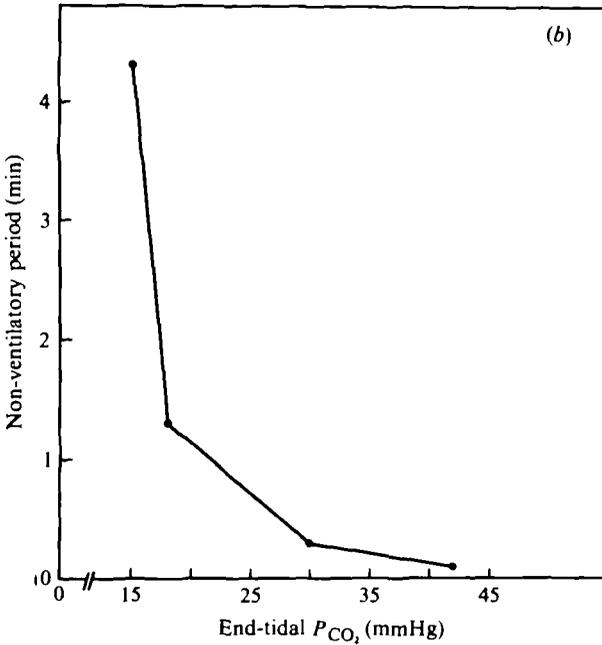


Fig. 8 (b). Relationship between end-tidal  $P_{CO_2}$  and duration of breath-holding (non-ventilatory period) in *Pelomedusa*.

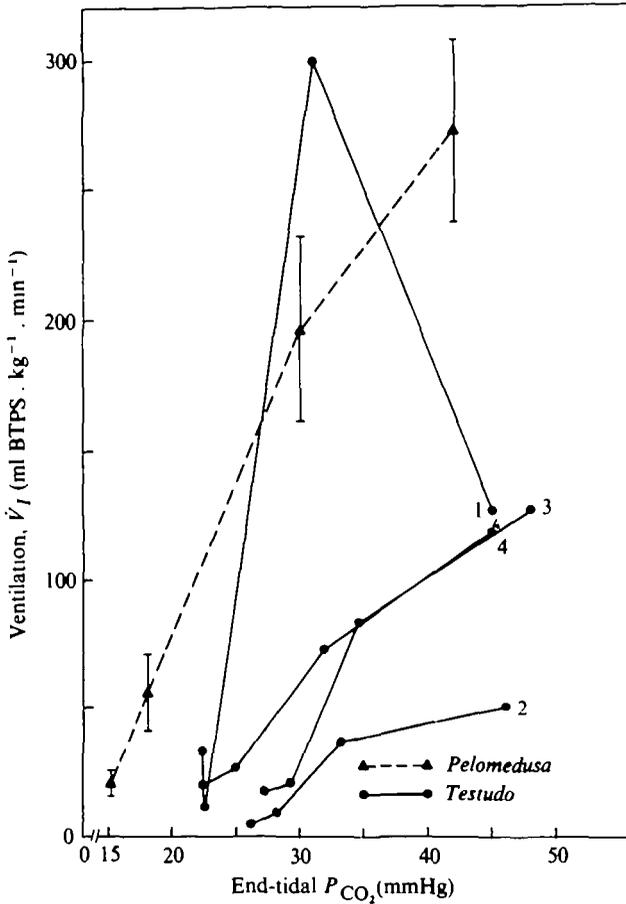


Fig. 9. Relationship between end-tidal  $P_{CO_2}$  and ventilation. The combination of animals and the symbols used are the same as in Fig. 7.

#### DISCUSSION

A contrast exists between the periodic breathing pattern of *Pelomedusa* and the alternation between single breaths and short periods of breath holding in *Testudo*, but these differences are not matched by any significant differences in the long term respiratory frequency. Weight-specific tidal volume in *Pelomedusa* exceeds that of *Testudo*. This leads to a higher weight-specific ventilation in agreement with the relative weights of the two species. The respiratory frequency and tidal volume of *Pelomedusa* are very similar to those reported for *Pseudemys* (Jackson, 1971). The high weight-specific tidal volume of diving turtles relative to that of *Testudo* will reduce the number of breaths needed to change gas composition in the lungs after a diving period. In snakes, the weight-specific ventilation of an aquatic species is less than for terrestrial species. The aquatic species practising long breath holds (up to 1 h) compensate for this by possessing much smaller air convection requirements (Glass & Johansen, 1976). Similar tendencies in chelonians are not obvious in the present data. Air convection requirements of *Pelomedusa* and *Testudo* at 25 °C are

not significantly different and close to the requirements of *Pseudemys* (Jackson, 1971). The lower end-tidal  $P_{\text{CO}_2}$  in *Pelomedusa* than in *Testudo* reflects aquatic  $\text{CO}_2$  elimination. Cutaneous respiration lowers blood  $P_{\text{CO}_2}$  and conversely, as stated by Dejours (1975): 'the more an animal is a pulmonary breather, the higher is its blood  $\text{CO}_2$  pressure'. Aquatic  $\text{CO}_2$  elimination also explains the relatively small fluctuations in lung and blood gas  $P_{\text{CO}_2}$  in spite of the occurrence of diving periods.

The hyperbolic relationship between inspired  $P_{\text{O}_2}$  and ventilation in both *Pelomedusa* and *Testudo* has earlier been reported for *Pseudemys* (Frankel *et al.* 1969; Jackson, 1973). In contrast Altland & Parker (1955) found that hypoxia influenced the pattern of breathing, but not the actual ventilation volume in *Terrapene carolina carolina*. The duration of the diving period has been shown to depend on  $P_{\text{IO}_2}$  in several species of turtles, *Terrapene* (Altland & Parker, 1955), *Chelydra serpentina* (Boyer, 1963) and *Chelys fimbriata* (Lenfant *et al.* 1970). The increase of ventilation in *Testudo* and *Pelomedusa* was not correlated with any consistent pattern of changes in frequency or tidal volume, as was similarly reported for *Pseudemys* (Jackson, 1973).

The fact that blood  $P_{\text{CO}_2}$  in *Pelomedusa* had not reached a stable value in relation to the  $P_{\text{CO}_2}$  of the inspired hypercapnic gas even after 30 min (Fig. 4) supports the statement by Wood & Lenfant (1976) that data on control of ventilation in reptiles should be based on measurement of gas tensions in the blood and the lungs, and not on inspired gas tensions alone. There is no obvious explanation for the low rate of increase of blood  $P_{\text{CO}_2}$  in *Pelomedusa*. Intracardiac right-to-left shunting of blood away from the lungs could be an explanation, but Burggren *et al.* (1977) actually found hypercapnia to stimulate lung perfusion. The  $\text{CO}_2$  diffusing capacity of the chelonian lung is low (Crawford *et al.* 1976) and thus could be partly responsible for the low rate of equilibration. A complication in the relationship between blood  $P_{\text{CO}_2}$  and ventilation is the fact that ventilation reached a maximum or even decreased, while blood  $P_{\text{CO}_2}$  continued to increase. Ventilation did not reach an absolute maximum limit at 4%  $\text{CO}_2$ , since inspiration of 6%  $\text{CO}_2$  could produce an additional increase of ventilation.

The results of the experiments on the relationship of ventilation to end-tidal  $P_{\text{CO}_2}$  are more clear. Ventilation and end-tidal  $P_{\text{CO}_2}$  increased at comparable rates in *Testudo*, except that ventilation reached a maximum level while end-tidal  $P_{\text{CO}_2}$  still increased, though very slowly. The fact that an increase of  $P_{\text{ETCO}_2}$  did not cease completely even after nearly 1 h of  $\text{CO}_2$  breathing indicates that the ventilation increase is inadequate for eliminating the  $\text{CO}_2$  produced by the animal in spite of an impressive increase of tidal volume. If lung volume of *Testudo pardalis* is comparable to a lung volume in *Testudo graeca* of 171 ml/kg (Crawford *et al.* 1976), then the ratio of tidal volume to total lung volume increases from 6% to slightly less than 30% during hypercapnia.

A simple relationship of ventilation to end-tidal  $P_{\text{CO}_2}$  was evident in *Pelomedusa* and *Testudo*, although complicated by some depression of ventilation at high  $\text{CO}_2$ . Ventilation increased in proportion to blood  $P_{\text{CO}_2}$  in *Pseudemys* (Jackson *et al.* 1974). The correlation between end-tidal  $P_{\text{CO}_2}$  and ventilation takes on interest in light of the recent evidence for the existence of intrapulmonary  $\text{CO}_2$ -sensitive receptors in the Tegu lizard (Gatz, Fedde, & Crawford 1975; Fedde, Kuhlmann & Scheid, 1977; Scheid, Kuhlmann & Fedde, 1977), and turtle (Milsom & Jones, 1976). The receptors

are both mechano- and  $\text{CO}_2$ -sensitive in the turtle, but not in the Tegu lizard in which the discharge rate of the receptors increases when  $P_{\text{CO}_2}$  in the lung gas is reduced. Additionally, a sudden reduction of lung  $P_{\text{CO}_2}$  induces a period of breath-holding in the Tegu lizard (Gatz *et al.* 1975). This response suggests that the receptors are involved in a positive ventilatory response to an increase of lung  $P_{\text{CO}_2}$ , which may explain the fairly simple relationships between end-tidal  $P_{\text{CO}_2}$  and ventilation seen in *Testudo* and *Pelomedusa*. However, the possible role of other peripheral receptor systems in reptiles has not been adequately investigated (Wood & Lenfant, 1976). Although there is still much to be learned about ventilation and the periodic breathing pattern in turtles, it is evident from the present data that the stimuli affecting ventilation in terrestrial and aquatic species are not fundamentally different. It is also evident that both  $P_{\text{CO}_2}$  and  $P_{\text{O}_2}$  may influence normal breathing in both *Testudo* and *Pelomedusa*. The influence of  $P_{\text{O}_2}$  on normoxic breathing is revealed by the fact that hyperoxia caused a depression of ventilation. The relative importance of  $P_{\text{O}_2}$  and  $P_{\text{CO}_2}$  in the control of normal breathing is difficult to analyse. Wood & Lenfant (1976) discuss the evidence that hypoxia is important in the control of breathing in chelonians. Although the present data show a very sensitive ventilatory response to hypercapnia in both the aquatic and the terrestrial species, it should be noted that fluctuations of blood and lung gas  $P_{\text{O}_2}$  in *Pelomedusa* are larger than fluctuations of  $P_{\text{CO}_2}$ , which tend to be decreased by aquatic  $\text{CO}_2$  elimination during normal breathing.

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